IL-6 Levels in Leprosy Patients with Reversal Reactions

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Submission date: 09-Aug-2019 09:27AM (UTC+0700)
Submission ID: 1158751807
File name: IL-6_levels_in_leprosy_patients.pdf (229.9K)
Word count: 1941
Character count: 11129

ORIGINAL ARTICLE

IL-6 Levels in Leprosy Patients with Reversal Reactions

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ABSTRACT

Background: Reversal reaction is type IV hypersensitivity reaction that frequently occurs in borderline leprosy and characterized as an acute inflammation of former skin lesion. Despite the lack of sufficient studies in literature on IL-6 in leprosy patients, theory supports IL-6 mast cell production in patients with reversal reaction. However, there were no studies which compared IL-6 produced by mast cell in RR leprosy patients.
Aim: To examine the IL-6 level in reversal reaction of leprosy and non-reactional leprosy.
Methods: 56 leprosy patients consist of 28 reversal reactions patients and 28 non-reactional leprosy were assessed for level of IL-6 in mast cell by immunohistochemistry staining. The IL-6 levels in both groups were analyzed using independent samples T test.
Results: IL-6 level was significantly increased in the reversal group compared to non-reversal group (mean ± SD, 3.68 ± 1.090 vs. 11.93 ± 2.372, p<0.001)
Conclusion: IL-6 level is associated with reversal reaction of leprosy.
Keyword: leprosy, IL-6

INTRODUCTION

Leprosy is a curable chronic infectious disease which can cause severe morbidity associated with disability. Leprosy is caused by intracellular obligate bacteria, *Mycobacterium leprae* that infects peripheral nerve, skin, oral mucous, upper respiratory airway, reticuloendothelial system, eyes, muscles, bone, testicles, and all of human organs other than central nervous system. Leprosy reactions are acute exacerbation manifested as, constitutional symptoms, disease activation and/or new skin efflorescence. There are 2 types of leprosy reactions: type 1 (Reversal Reaction/RR) and type 2 (Erythema Nodosum Leprosum/ENL)[1].

Reversal reaction is type IV hypersensitivity reaction that frequently occurs in borderline leprosy as a result of cellular immune response to *M. leprae* antigen and characterized as an acute inflammation of former skin lesion. Approximately 95% of RR occurs simultaneously with diagnosis confirmation or during multi drug treatment (MDT). RR commonly appears on the first six months of treatment, particularly in BT and BB leprosy. It can also be found in BL leprosy with longer interval during MDT. Clinical manifestation of RR includes abrupt increased in number and more active lesion with/ or without ulceration, edema, neuritis, and permanent nerve damage. Bacterial index (BI) is frequently negative or remarkably decreased in RR patients. Leprosy patients commonly complain about skin lesion enlargement which is aesthetically disturbing. This causes treatment withdrawal since patients consider it as a treatment failure[1,2].

Interleukin-6 (IL-6) is a cytokine produced by mast cell along with other cells, as a response to varying stimuli. This is a multifunctional cytokine that is involved in immune response regulation, acute phase reactions, inflammation and hematopoietic growth. Despite the lack of sufficient studies in literature on IL-6 in leprosy patients, theory supports IL-6 mast cell production in patients with reversal reaction[3,4].

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IL-6 is synthesized mainly in the presence of IL-1, TNF- α, and lipopolysaccharide. IL-6 plays role in innate and adaptive immune response, particularly in acute phase reactions. IL-6 stimulates T lymphocyte, contributes in B lymphocyte production and antibody production via Th2-cell-mediated immune response. Previous studies have successfully shown that monocyte production of IL-6 is higher in RR patients compared to non-reactional leprosy patients.[5] However, there were no studies which compared IL-6 produced by mast cell in RR leprosy patients. Our hypothesis is that in reversal reactions there is increasing number of IL-6 produced by mast cells that have a role in the pathophysiology of leprosy reaction. Thus, this study aimed to compare IL-6 level in RR and non-RR.

MATERIALS AND METHODS

Research design in this study was analytic observational with cross sectional study. Subjects were multibacillary leprosy patients being treated at the Donoharjo Hospital, Jepara. Selection of subject has done by consecutive sampling with double blind method. Participants were multibacillary leprosy patients who met WHO criteria and aged between 20-60 years old. Participants were divided into two groups; leprosy patients with reversal reaction group and leprosy patients without reversal reaction. Written informed consents were obtained from all participants. Exclusion criteria was included pregnancy and other acute inflammatory diseases. Based on the sample calculations, total subjects in this study were 56 samples: leprosy patients with reversal reaction group (n=28) and leprosy patients without reversal reaction (n=28). IL-6 levels were measured by immunohistochemistry staining using monoclonal antibodies to IL-6 (Leica Biotech). It was visualized by DAB and calculated by observing 20 fields of view in 1000x magnifications (Fig. 1).

The IL-6 levels in both groups respectively were analyzed using independent samples T test. The data were analyzed using normality and homogenity test for age and occupation. Confounding factors in this study were controlled by randomization proccess. The data were significance if p <0,05 with confidence interval 95%. This

study was approved by Ethics Committee of Faculty of Medicine, University of Airlangga.

RESULTS

This study showed a significant increase (p = 0.000) in the expression of IL-6 in patients with reversal reaction group compared to non-reactional leprosy group. (Fig. 2).

Table 1: IL-6 expressed by mast cells in patients with reversal reaction group compared to non-reactional leprosy (non-RR) group

Groups	IL-6 (Mean ± SD)	p
RR	3.68 ± 1.090	0.000[a]
Non-RR	11.93 ± 2.372	

[a]Mann-Whitney test

Fig.1: This picture shows positively reactive mast cell to anti-IL-6 through immunohistochemistry staining, x400. Figure A: non-RR group. Figure B: RR group. Black arrow indicates double stain to anti-IL-6, red arrow indicates nonreactive mast cell.

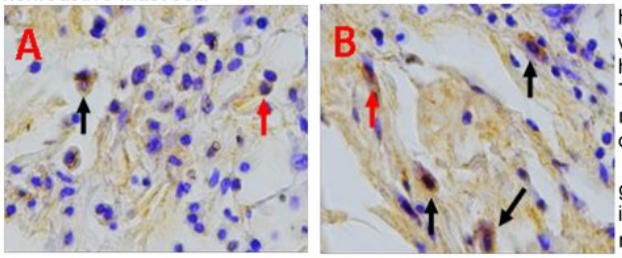
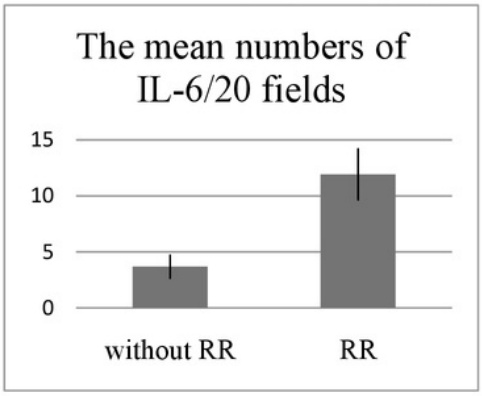


Fig. 2: The histogram above shows increased expression of IL-6 produce by mast cell in patients with reversal reaction group compared to non-reactional leprosy (non-RR) group (p = 0.000)



DISCUSSION

Reversal reaction was marked by delayed hypersensitivity to *M. leprae* antigen (Gell & Coombs type IV reaction) and abrupt increase in immune cell response.[6] Theoretically, reversal reactions was seen as germs destruction was a positive thing as it occurred massively. However, it was clinically dissatisfying because of the acute inflammatory reaction which was aesthetically disturbing. Reversal reaction affected 20-30% of leprosy patients. RR occurred more frequent than ENL. Clinical manifestation of RR was acute inflammation of existing lesions, which could be erythematous, enlarged or transformed into infiltrates[5].

In this study, IL-6 levels were examined by comparing IL-6 mast cell production in patient with RR compared to non-RR patients. There was a significant difference (p = 000) of IL-6 in both groups.

Reversal reaction was associated with cell-mediated immunity characterized with granuloma expansion, oedema, immune-inflammatory cells recruitment and cytokines (TNF-α, IL-1. IL-6, dan IL-8). Reversal reaction was highly affected by mast cell degranulation of pro-inflammatory cytokines. Studies showed IL-6, IL-8, and TNF-α increase in leprosy reversal reaction. IL-6, IL-8, and TNF-α pro-inflammatory effect in leprosy reversal reaction marked an increase in mast cell activity through the ongoing inflammation process and exacerbated existing lesion: lesion abruptly became more plenty and more active with or without ulceration, oedema, neuritis and permanent nerve impairment[7,8].

Leprosy patients with reversal reaction were proved to have strong antibody response to *M. leprae* antigen which was an antigen protein weighed 70-kDa and identified to have 47% homologous sequence with Hsp-70 in humans. This phenomenon was known as antigenic mimicry: the most rational explanation to reversal reaction-related clinical and immunopathological manifestations[9].

Hsp-70 release in extracellular environment would generate immune response acting as *red flag* to the immune system. Hsp-70 increase triggered defense mechanism to pathogens through TNF-α production in patients with reversal reactions. Hsp-70 had been proved to act as a potent trigger in IL-6 secretion. However, it had not been fully understood whether Hsp-70 generates its own attempt to protect tissues from *M. leprae* antigen through inflammatory response or the presence of Hsp-70 itself created immunopathological reactions related to the occurrence of reversal reactions. In this regard, Hsp-70 facilitated inflammatory response by inducing mast cell activation. Mortaz *et al* had proved that Hsp-70 plays role in mast cell degranulation. Heat shock induction to bone marrow derived mast cell (BMCC) triggered Hsp-70 release to extracellular environment and stimulates TNF-α and IL-6 production through TLR4 receptor pathway found on mast cell surface[10,11].

Previous studies had successfully shown a significantly higher monocyte production of IL-6 in patient with RR compared to non-RR patients.[5] IL-6 had both anti-inflammatory and pro-inflammatory properties and was a strong inductor of acute phase protein reaction. IL-6 had been used as systemic activation of pro-inflammatory reaction. As a pro-inflammatory cytokines, IL-6 production was induced by TNF-α and IL-1[3,4].

This study showed a significant increase in mast cell IL-6 production in leprosy patients with reversal reactions. This result was in accordance with proposed hypotheses. In this study, there were several limitations that could confound the result of the study. Cohort design was better to evaluate an actual effect, however it was highly unethical to wait for a patient to develop a reaction, and therefore cross-sectional design was chosen to examine the association among variables. There were plenty other variables to be explored regarding research in leprosy reversal reactions but were not looked into because

of financial limitations. Moreover, the lack of sufficient literature on variables related to patients with leprosy made it difficult to determine base line data in each of those variables.

Acknowledgments: The authors would like to express our gratitude to Department of Dermatology and Venereology, Diponegoro University and Donorejo Hospital, Jepara.

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PAGE 1

PAGE 2

PAGE 3